Fluorographs of \( ^{35} \)S-methionine-labelled muscle cell proteins at various stages of differentiation

Days in vitro after the addition of medium containing horse serum were: A, 1; B, 6; C, 10; D, 14. Polypeptides whose synthesis is increased after fusion are marked by upward pointing arrowheads in B, C, D, and those which decrease are marked by downward-pointing arrowheads in A. The large spot to the left of centre is actin, below this to the left two spots are arrowed in C which are presumed to be \( \alpha \) - and \( \beta \)-tropomyosin. Myosin heavy chain is probably the streak at the top of the gel, and the spots arrowed in D are thought to be myosin light chains. All gels are oriented with the acidic site to the left. 2000000 d.p.m. were loaded per gel. First dimension: pH4-9 mix BASO, 1600 Vh; second dimension 12% (w/v) polyacrylamide, 0.1% sodium dodecyl sulphate.

Certain proteins on two-dimensional gels do show variation in expression in dystrophic cultures, but have been difficult to characterize properly because of culture variations. It is hoped that the use of cell clones which appear phenotypically similar with respect to various antigenic markers will help in the elucidation of genuine disease markers.

We thank Dr. F. S. Walsh (Institute of Neurology, London), for the gift of the monoclonal antibodies, and the Muscular Dystrophy Group of Great Britain and Northern Ireland for the financial support of this work.

STUART D. M. WATTS and ADRIAN M. ATKINS
Biochemical Microbiology Department,
The Wellcome Research Laboratories, Beckenham,
Kent BR3 3BS, U.K.

Mechanism-based inactivation of GABA-transferase from a nematode parasite

STUART D. M. WATTS and ADRIAN M. ATKINS
Biochemical Microbiology Department,
The Wellcome Research Laboratories, Beckenham,
Kent BR3 3BS, U.K.

Fig. 1. Fluorographs of \( ^{35} \)S-methionine-labelled muscle cell proteins at various stages of differentiation

Fig. 2. Immunofluorescence of human muscle cells

(a) Phase contrast of a secondary muscle culture and indirect immunofluorescence of the same field with muscle-specific antibody 5.1H11. (b) The variable reactivities of three different muscle clones B2, C6 and A3 with three monoclonal antibodies 5.1H11, 24.1D5 and 16.3A5.

Mechanism-based inactivation of GABA-transporter from a nematode parasite

One of the most effective known inhibitors of GABA-T (4-aminobutyrate: 2-oxoglutarate aminotransferase; EC 2.6.1.19) is hydrazinopropionic acid (HPA) (Van Gelder, 1968). We report here that this compound inhibits the enzyme from a nematode parasite of rats, *Nippostrongylus brasiliensis*, in a mechanism-based fashion rather than as a non-specific carbonyl-trapping reagent.

Nematode GABA-T activity was assayed by use of a radiometric technique according to Watts & Atkins (1983, 1984) at substrate concentrations of 0.33 mM (GABA) and 0.57 mM (2-oxoglutarate). Inclusion of HPA in a buffered preincubation mixture containing enzyme and pyridoxal phosphate (PLP) but no substrate for 15 min at 2.6.1.19 is hydrazinopropionic acid (HPA) (Van Gelder, 1968).
37°C resulted in a marked inhibition of activity (in a volume of 70 µl, the mixture contained inhibitor, 58 mM-potassium phosphate, 5% Triton X-100, 23 µM-aminopterin, 100 µM hydroxylamine, 70 µM-EDTA, 65 µM-PLP) which was revealed upon addition of GABA and 2-oxoglutarate to make the final incubation volume up to 100 µl. The molar concentration of HPA, in the preincubation, required to give 50% inhibition (IC₅₀) was 3.3 × 10⁻⁵. In comparison, the IC₅₀ values with several other PLP-dependent enzymes were: rat brain GABA-T, 2.0 × 10⁻⁵; pig heart alanine aminotransferase, 5.8 × 10⁻⁵; Escherichia coli glutamic acid decarboxylase, 2.6 × 10⁻⁵. In view of the large differences in potency against these enzymes it seemed unlikely that HPA was simply functioning as a carbonyl-trapping agent. Exposure of HPA to a 1000-fold greater concentration of PLP for 15 min at 37°C before commencing the usual preincubation with GABA-T did not significantly reduce the efficacy of HPA.

Further investigations revealed, when substrates and inhibitors were added to GABA-T incubations simultaneously, that there existed a competition between GABA and HPA. If GABA (0.49 mM) was added to the 15 min preincubution mixture of GABA-T simultaneously with 7 nM-HPA the inhibition revealed after a subsequent 30 min incubation at 37°C was reduced from 100% (preincubation without GABA) to insignificant levels relative to controls. Addition of 0.81 mM-2-oxoglutarate did not protect the enzyme from inhibition.

When HPA was added to GABA-T at 37°C before addition of GABA there was a progressive inhibition up to about 10 min elapsed preincubation time. The degree of inhibition was dependent upon the HPA concentration and during this initial period it could be prevented by secondary addition of GABA. Thereafter, GABA was ineffective in relieving any inhibition. For a given concentration of HPA, the inhibition did not worsen after this initial period even if preincubation was prolonged for 30 min.

A more detailed analysis of this time-dependent initial phase of the inhibition by HPA is presented in Fig. 1(a). From the information above, it is clear that after addition of GABA no further inhibition would occur during the 30 min incubation to complicate analysis. These results were analysed according to Kitz & Wilson (1962) for time-dependent inhibition and gave the kinetic parameters (Kᵢ = 173.9 nM and the first-order rate constant for inactivation = 0.045 s⁻¹) (Fig. 1b).

The present work demonstrates that HPA: (i) is a specific and potent inhibitor of GABA-T, (ii) competes with GABA but not 2-oxoglutarate for the active site of the enzyme, (iii) causes a time-dependent loss of enzyme activity that is reversible, initially, but then becomes irreversible.


![Graph](image)

**Fig. 1. Analysis of time-dependent inhibition of N. brasiliensis GABA-T by HPA**

(a) GABA-T was preincubated alone with HPA. Reaction conditions were as described except that PLP was omitted from all buffers during both preparation of the enzyme and incubations. Sufficient GABA-T was utilized to give around 2500 net d.p.m. in control samples (equivalent to approx. 10 µg of protein). Lines were fitted by linear regression and the ordinate intercept taken as 100% activity. •, Control. HPA concentration (nM): ○ 2.9; ▲, 4.3; △, 7.1; ■, 14.3. (b) Secondary reciprocal plot of slopes (Kᵢ) from (a) corrected for control slope versus HPA concentration; linear regression correlation coefficient = 0.997.


**The induction of an arginine-metabolizing enzyme in *Methanobrevibacter smithii* PS**

NIGEL L. BLUMSOM, ADRIENNE HEALY and FIONA HOGAN

*Department of Biochemistry Medical Biology Centre, Queen’s University, Belfast BT9 7BL N. Ireland, U.K.*

*Methodobrevibacter smithii* is a member of the proposed third kingdom of life, the Archaebacteria, and it has been shown that significant differences exist between organisms of this kingdom and the Eubacteria and Eukaryotes, both as regards their macromolecules, coenzymes and metabolic pathways (Kandler, 1982). We have isolated, purified and partially characterized three proteinases from this organism to ascertain features which differ from previously described proteinases (N. L. Blumson,